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CERTIFICATE

This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 5 February 2004 with an application for Letters Patent number 530972 made by Frontin-Rollett, Andrew.

I further certify that pursuant to a claim under Section 24(1) of the Patents Act 1953, a direction was given that the application proceed in the name of Select XY Limited.

Dated 4 February 2005.

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530972

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Patents Act 1953

PROVISIONAL SPECIFICATION

Title: A Method And Apparatus For Selecting Cells

I, ***Frontin-Rollet, Andrew***,
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do hereby declare this invention to be described in the following statement :

PF04.JWP : sc

FEE CODE – 1010

A METHOD AND APPARATUS FOR SELECTING CELLS

FIELD OF INVENTION

The present invention generally relates to a method and apparatus for selecting desired cells, or parts of cells, preferably, desired sperm cells and more particularly relates to a method and apparatus for orientating, selecting and retaining desired cells or parts of cells.

BACKGROUND OF THE INVENTON

There has been a long felt need for a reliable, qualitative and cost-effective method for selecting sperm which may be used to produce animals of a desired sex.

In particular, in the livestock industry farmers or breeders require cows, pigs, sheep, goats, deer, buffalo, horses, etc which are of a preferred sex. For example, bulls are of limited use to a dairy farmer, whereas pig farmers have long been aware that the female pig grows at a faster rate than their male counterparts.

Similarly, cattle and sheep farmers understand only too well that the males of these species produce meat at a faster rate than females.

In mammals the egg carries only the X chromosome whereas the sperm carries either an X or a Y chromosome. The sex of progeny is therefore determined by the sperm cell. When a sperm and an egg are combined and the sperm carries the X chromosome the offspring is female (XX). However, if the sperm carries the Y chromosome, once combined with the X chromosome carried by the female the resultant offspring will be male (XY).

In sperm there is a known difference in DNA content between the X (larger) and the Y (smaller) sperm of for example 3.4% in pigs, 3.8% in cattle and 4.2% in sheep. This measurable difference can be used to determine the sex of the sperm, that is, if it is an X chromosome (female) or if it is a Y chromosome (male) bearing sperm.

The prior art discusses and provides for methods for sorting mammalian sperm into X and Y populations. However the only reliable methods are based upon the measurement of the DNA content of individual sperm. These methods invariably use fluorescence measurement to detect what are essentially small differences between the X and Y sperm, wherein the sperm pass single file through a system which measures the DNA content of individual sperm.

Some techniques have been expanded to use a bevelled sample injection tip and a second fluorescence detector in a forward position. The second fluorescence detector is adapted to determine the orientation of flat oval shaped sperm with respect to the first detector as they pass through the system..

In both cases the magnitude of fluorescence is being measured.

Further adaptations allow for those unwanted sperm to be gated and pass through as waste and discarded.

The prior art therefore describes a flow cytometric system, which requires two separate measurements of the magnitude of fluorescence of the sperm cell, one to determine the sex of the sperm, the other to determine the sperm's orientation. Those skilled in the art would recognise that due to the morphology of sperm cells (flat ovoid shape) and extremely high refractive indices, it is not possible to accurately measure the DNA content of sperm unless said sperm are correctly oriented to the DNA fluorescence detector.

This method of analysis is expensive – and do not always provide for routine efficiencies much in excess of 80%, although 95% efficiencies have been reported.

Surprisingly, the present inventor has found that a process wherein the orientation of a sperm cell is determined by passing light using optical phase contrast techniques through a sperm cell of interest provides for improved efficiencies and increased reliability in the results obtained. In other words orientation of sperm cells – the correct orientation defining whether a result should be accepted for further analysis – can be determined by measuring non-fluorescent light emitted by a sperm cell.

The measurement of non-fluorescent light has never before been considered as a means to determine the orientation of cells.

Surprisingly, the inventor has further found that there is no need for the cells of interest to be encapsulated or confined within an electrically charged medium during the analysis and collection phase of the process. Previously, once the DNA content of a sperm cell had been determined, the cells were encapsulated in a droplet to which is appended an electric charge, the charge being dependent on the cells DNA X or Y sex chromosome content. The droplets were then separated based upon the charge they received. The present invention simply selects those cells having a desired DNA sex chromosome based upon predetermined parameters programmed in the analyser. If the criteria are met the cell is collected and retained. If the criteria are not met the cell is eliminated by a process of ablative photodecomposition, generally by exposure to a laser.

This invention also teaches the use of a rectangular testing zone located downstream of an orienting nozzle. A cell emerging from the orienting nozzle can be maintained at the correct radial orientation to allow for accurate analysis. The substantially rectangular configuration of the testing zone of the invention has been found to provide for superior accuracy and more reliability in the results obtained. Previous testing processes maintained the cell being measured (although generally correctly aligned) in a circular cross sectional fluid stream or liquid droplet which is of an essentially elliptical or circular configuration as the cell emerged from the nozzle. This configuration, although allowing for increased cell flow rates of a desired orientation, also allows for inaccuracies due to light being refracted from the curved surfaces of the fluid stream or droplets being measured.
Importantly, this rectangular testing zone provides for four flat surfaces. The improvement results in a significant reduction in unwanted refracted light which when curved surfaces are used thus eliminated false readings. As such the provision of four flat surfaces provides for a much-improved reliability over previously disclosed systems.

The present invention comprises therefore at least three components, the aspects of which will be outlined later in greater depth. Firstly, the invention uses phase contrast optics to determine a cells

orientation. Secondly, the invention makes no requirement for the cells of interest to be encapsulated in droplets or otherwise to enable desired cells to be sorted from those that are not wanted. Thirdly, the use of a substantially rectangular testing zone reduces the effects measurement of unwanted light has on the process.

The above features therefore provide significant and surprising advantages over existing cell selection/sorting processes and in particular those processes directed to the sexing of sperm cells.

OBJECT OF THE INVENTION

It is an object of the present invention to provide an improved method and/or apparatus for selecting desired cells, or parts of cells, or is one which will obviate or minimise the foregoing disadvantages or will at least provide the public with a useful choice.

STATEMENT OF THE INVENTION

Accordingly, a first aspect of the invention provides for a method of determining the orientation of a cell in a process wherein said orientation is used to allow for the determination of cell differences due to size, mass, volume or density and whereby the orientation of the cell is determined by measuring non-fluorescent light.

Preferably, the orientation is determined by measuring light using a band pass filter to exclude all light other than from the phase contrast light source/condenser.

Preferably, the method for determining the orientation of the cell does not require the cell to be encapsulated within a droplet.

Preferably, the method for determining the orientation of the cell is used in tandem with a method for measuring the DNA content of the cell.

Preferably, the method for determining the orientation of the cell is used simultaneously with a method for measuring the DNA content of the cell.

Preferably, the method for determining the orientation of the cell is used in a method for selecting sperm of a desired chromosome complement.

Preferably, the method for determining the orientation of the cell is used in a method for selecting X chromosome bearing sperm from Y chromosome bearing sperm.

Accordingly, a second aspect of the invention provides for a method of selecting a desired cell, or parts of a cell, the method having the following steps:

(i) passing suitably maintained cells from a sample of cells of interest into a testing zone,
(ii) exposing said cell sample of interest to a first light source having a first wavelength,
(iii) exposing said cell sample of interest to a second different light source of a second different wavelength,
(iv) collecting light energy emitted at (ii) and (iii) above,
(v) analysing the light collected at (iv) to determine whether the desired predetermined parameters are met,
(vi) selecting for those cells, or part of cells, which meet said desired parameters,
(vii) collecting the selected cells in a suitable viability maintenance medium, and/or
(viii) eliminating those unwanted cells, or parts of cells, as waste.

Preferably, the cells are sperm cells

Preferably, the sperm cells are stained with a fluorochrome.

Preferably, the first light source is an excitation light source.

Preferably, the first light source is adapted to measure fluorescent light.

Preferably, the first light source is used to analyse the DNA content of a sperm cell.

Preferably, the fluorochrome is selected from SYBR green I, SYBR green II, SYBR gold, and Bisbenzimide H33342

Preferably, the second light source is used to determine the orientation of the cell.

Preferably, the second different light source uses phase contrast optics.

Preferably, the cell is simultaneously exposed to said first light energy and second different light energy.

Preferably, the cell is passed through an orientation device wherein the orientation of the cell is hydrodynamically oriented to achieve a uniform radial geometry with respect to the detector(s)

Preferably, the testing zone is a rectangular receiving area adapted to maintain the orientation of single cells, most preferably sperm cells during analysis and/or selection.

Preferably, the cells to be tested are delivered to the rectangular testing zone at a flow rate above 1,000 cells per second, preferably 1,500 to 10,000 cells per second.

A third aspect of the invention provides for an apparatus for selecting a desired cell, or a parts of a cell, the apparatus comprising:

(i) a means for passing suitably maintained cells from a sample of cells of interest into a testing zone,

(ii) a means of exposing said cell sample of interest to a first light source having a first wavelength,

(iii) a means of exposing said cell sample of interest to a second different light source having a different wavelength,

(iv) separate means for collecting and amplifying light emitted by said sample at (ii) and (iii)

(v) a means for analysing the data collected by separate means (iv) to determine whether desired predetermined parameters are met,

(vi) a means for selecting, collecting and maintaining cells in viable condition meeting said desired predetermined parameters, and/or

(vii) a means for eliminating, those unwanted cells, or parts of cells, as waste.

Preferably, said first light source is a laser.

Preferably, said first light source is adapted to analyse the DNA content of a cell.

Preferably, said second light source is derived from phase contrast optics.

Preferably, said second light source is adapted to determine the orientation of a cell.

Preferably, said means for collecting light emitted said sample after exposure to said first light source comprises a an objective.

Preferably, said means for collecting light emitted by said sample after exposure to said second light source is an optical detector adapted to collect light energy of a non-flourescent wavelength.

Preferably, said analysis and selecting means is a multi-channel analyser or computer programmed with suitably developed computer software.

A fourth aspect of the invention provides for a delivery device adapted to deliver individual cells, or parts of cells, to a test zone, the delivery device comprising:
an elongated tube defined at one end by a nozzle, the nozzle adapted to deliver cells, or parts of a cells to the testing zone in a correct orientation suitable for testing,

adjacent to said nozzle, a rectangular test area adapted to maintain said cells at the correct orientation for testing,
and wherein in use, as the cells pass through the tube and out of the orientation nozzle said device is capable of maintaining the orientation of individual cells in a position which allows for an individual cell to pass through a first light source having a first wavelength and light emitted by said cell to be detected and analysed, and

which allows for a said cell to pass through a second different light source having a second different wavelength, to be detected and analysed.

A fifth aspect of the invention further provides for a method of selecting a desired sperm cell, or part of a sperm cell, the method having the following steps:

(1) staining intact, viable sperm collected from a male mammal with a suitable fluorescent dye, such that the DNA takes up the fluorescent dye uniformly,

(2) maintaining the stained sperm in an suitable maintenance medium sufficient to maintain the sperm and/or contained DNA within the cell in a viable condition,

(3) passing the maintenance medium containing the sperm before a suitable excitation light source causing the stained DNA to fluoresce,

(4) passing the maintenance medium containing the sperm through both a means for measuring the fluorescence of the stained DNA and a means for detecting the orientation of the sperm,

(5) collecting light energy emitted by said sperm cell, converting the light energy into electrical signals and analysing the electrical signals via a multi-channel analyser or suitably programmed CPU,

(6) selecting those sperm cells, or parts of sperm cells meeting desired predetermined criteria, and

(7) a means for eliminating those cells, or parts of cells, which fail to meet the desired predetermined criteria .

Detailed Description of the Invention

The following examples are illustrative only and, where specific integers are mentioned which have been known equivalents, such equivalents are deemed to be incorporated herein as if individually set forth.

The examples describe preferred embodiments only are meant to be in no way limiting.

The present application has particular relevance in the selection of sperm cells carrying a desired sex chromosome. The ability to provide for populations having X chromosome bearing sperm or Y chromosome bearing sperm at a purity of 95% or even up to 99% is now achievable.

Brief Description of the Drawings

Various other objects and features and attendant advantages of the invention will become more fully appreciated as the same becomes understood in conjunction with the accompanying drawings, in which like reference characters designate the same or similar parts throughout several views, and wherein

Figures 1A is a schematic showing the Phase A and Phase B.

Figure 1B is a schematic overview illustrating various light paths interact in the testing zone

Figure 2 illustrates the delivery tube and rectangular testing zone

Figure 3 is top view of testing zone to which has been delivered a cell of interest.

Figure 4 is a flow chart of the process

Example 1

Turning now to figures 1 to 4, the various apparatus used and method steps involved in the process are described in detail.

The live sperm the subject of the selection, are collected by standard collection techniques and maintained in a suitable medium such as a Tris buffer. The DNA within the cells is stained with a non-toxic fluorochrome, preferably SYBR green I, SYBR green II, SYBR gold or Bisbenzimide H 33342. Intact stained sperm are then subjected to a fluorescence excitation laser, the preferred excitation wavelength being about 488-497mm, the wavelength being dependent on the fluorochome being used. Signals emitted are collected and, via a photomultiplier tube (PMT) and CPU/analyser, the readings taken are analysed. If after analysis the sperm cell under investigation

meets desired criteria the sperm cell is selected, collected and maintained in an appropriate maintenance fluid – for later use in insemination.
Those cells that do not meet the predetermined criteria are eliminated by a process of ablative photo decomposition, generally by exposure to a laser.

Referring to figure 1, individual sperm cells are allowed to pass in single file through a nozzle (8) (see figure 2) and into a receiving area (10). The receiving area (10) (see figure 3) is generally rectangular in shape and is of a dimension which allows for individual sperm cells to be accommodated and their orientation maintained for analysis. The flow of sperm cells is continuous throughout the process. Flow rates of between 1,000-5,000 sperm per second are contemplated, although flow rates of 35,000 per second are not unknown.

The analysis and selection of desired sperm comprises two phases. The phases are preferably conducted simultaneously, but not necessarily. There may be occasions when the phases are concomitant.

In phase A, an individual sperm cell has previously been stained with a fluorochrome, The fluorochrome binds to the DNA. The amount of fluorochrome that binds to the DNA is dependent on the amount of DNA present. Given that an X chromosome contains more DNA than a Y chromosome, a female sperm (X) take up a measurable amount of more fluorochrome than does a male sperm (Y).

The more fluorochrome taken up the more fluorescence is emitted, and the differences between individually fluorescing cells able to be measured.

Individual sperm cells pass through the rectangular receiving area (10) and are exposed to a fluorochrome excitation light source (15) in the form of a laser beam. The fluorochrome bound to the DNA is excited and fluoresces. The fluorescence is collected through a phase contrast objective (11) travel through a dichromic mirror (12), filtered by an appropriate band pass filter (25) and amplified by a PMT (14), digitised and forwarded to a CPU/analyser (19) for analysis.

Phase B operates simultaneously with Phase A. Here, individual sperm arriving at the rectangular receiving area (10) are subjected to a phase contrast optical system (22) whereby non-fluorescent light (60) emitted from the sperm being tested is collected through a phase contrast objective (16), and passed through a band pass filter (23) to exclude any residual fluorescent light or any residual light occurring in a band width (450nm – 550 nm). The refracted light is pre-amplified (17), optionally filtered through a further filter (24) to exclude light emitted from the ablative photo decomposition laser amplified, digitised, measured with a PMT (18) and transported to a CPU/analyser (19) suitably programmed for analysis. Once analysis is completed those cells not meeting predetermined criteria are eliminated via an off ablative photodecomposition device (laser). The laser (20) is located downstream of the measurement process and is controlled by the CPU/analyser.

Figure 1A is described by the following:

1. cell
10. testing zone (rectangularly configured to provide four substantially flat surfaces)
11. phase contrast objective
12. dichromic mirror
13. pre-amplifier
14. PMT
15. Fluorochrome excitation energy source
16. Phase contrast objective
17. Pre-amplifier
18. PMT
19. Analyser/CPU
20. Ablative photodecomposition device (laser)
22. phase contrast optics
23. band pass filter
24. band pass filter (to exclude aberrant light from 20)
25. band pass filter

A means comprising a second geometric axial motion system allowing gentle deceleration of desired cells to be collected via a pipette or the like and maintained in a suitable medium for later use is also contemplated. The collection means is located downstream of the delivery device and the testing zone and will act much like a groyne in a river system.

Example 2

The mechanism by which suitably stained and intact sperm are provided for testing as described previously is illustrated by the device shown in figures 2 and 3.

The device is defined by an elongate tube (5) which at one end tapers to a nozzle (8).

Downstream of the nozzle is a rectangular testing zone (10). The testing zone (10) is invariably a tube that is of a dimension which accommodates and maintains the orientation of individual cells which allows for testing, analysis and consequent selection of those cells meeting desired criteria.

In particular, the testing zone (10) maintains the orientation of delicate cells so as to pass the cells in single file, through a first light source. The first light source is preferably derived from a laser. Sperm cells stained with an appropriate fluorochrome, such as SYBR I, SYBR II, SYBR gold Bisbenzimide H 33342, are excited, fluoresce and the magnitude of fluorescence is measured.

Simultaneous to the above, sperm is exposed to a second different light source, such as light derived from an optical phasecontrast system. This light source is projected horizontal to the first light source. The sperm being tested emits light. The light is captured, amplified and analysed by a multi-channel analyser or appropriate computer analysis.

The fluorescence emitted as the sperm passes through the first light source is used to identify whether the sperm carries an X or Y chromosome. The non-fluorescent light refracted by the sperm cell provides for an improved determination of its orientation.

The reader will be aware that only those cells oriented correctly can be used to predict with accuracy the DNA content and therefore the sex characteristics of the sperm.

All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

Alternative features serving the same, equivalent or similar purpose may replace each feature disclosed in this specification (including any accompanying claims, abstract and drawings), unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features.

The invention is not restricted to the details of the foregoing embodiment(s). The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

ADVANTAGES

The present invention has one or more of the following advantages:

- comparatively inexpensive
- allows for impressive sample flow rates
- provides increased efficiencies
- easier mechanical operation
- improved reliability
- improved viability of selected samples
- increased sample orientation dependability
- increased purity of collected sample

Variations

Some preferred aspects of the invention have been described and illustrated by way of example, but it will be appreciated that other variations of and modifications to the invention can take place without departing therefrom.

For example, it is envisaged that although the specification is predominantly directed to the sorting of sperm cells into X and Y populations the possibility of selecting for example white blood cells from a blood sample or gram negative bacteria from a suitably prepared sample is contemplated.

The use of such a method to isolate and select for viruses of interest is also an option.

The skilled reader will also instantly realise that the use of filters to exclude light energy of unwanted wavelength may also vary depending on the sample under investigation.

Throughout the description and claims of this specification the word "comprise" and variations of that word, such as "comprises" and "comprising", are not intended to exclude other additives, components, integers or steps.

PIPERS

Attorneys for

ANDREW FRONTIN-ROLLET

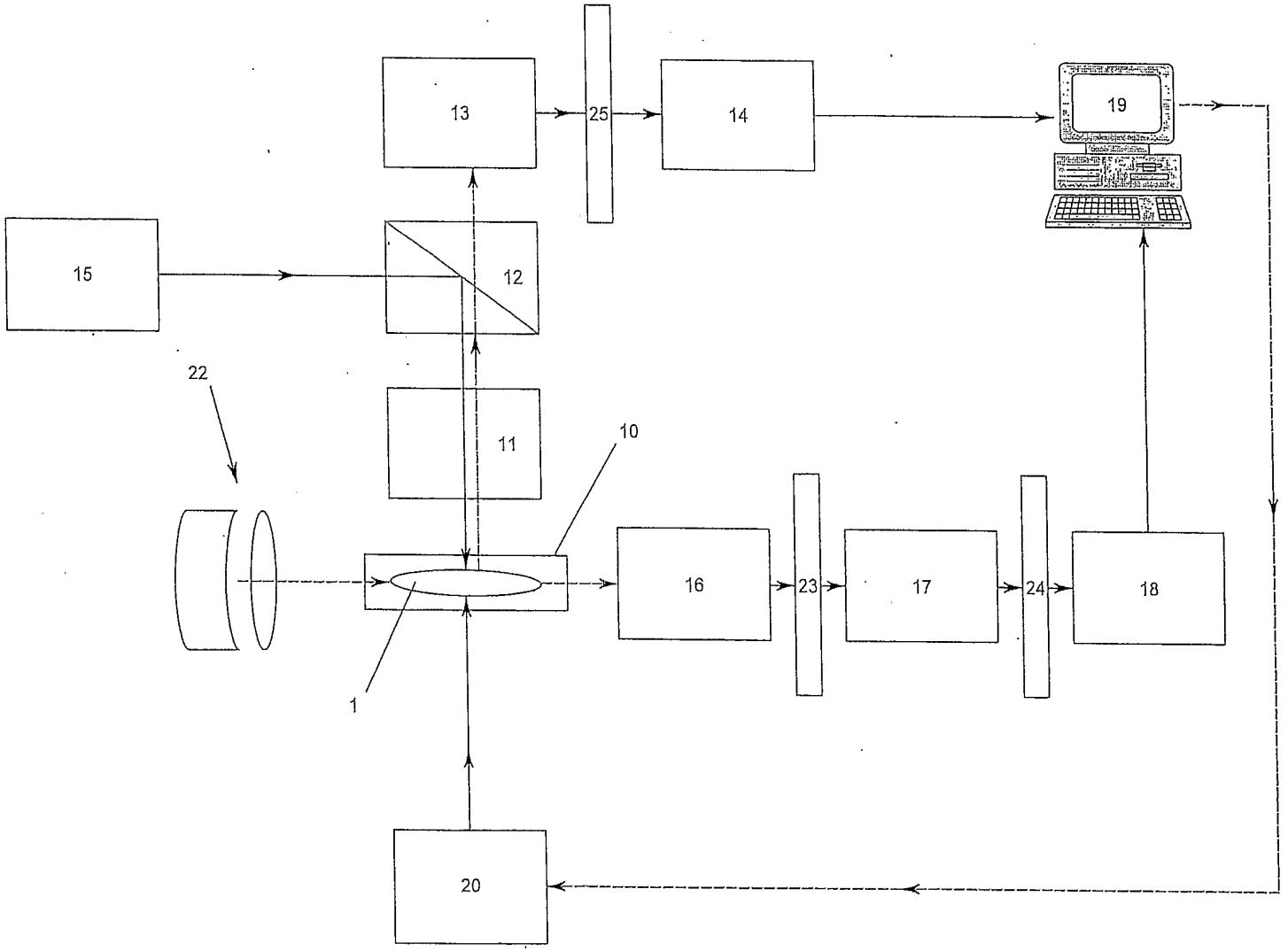

FIGURE 1A

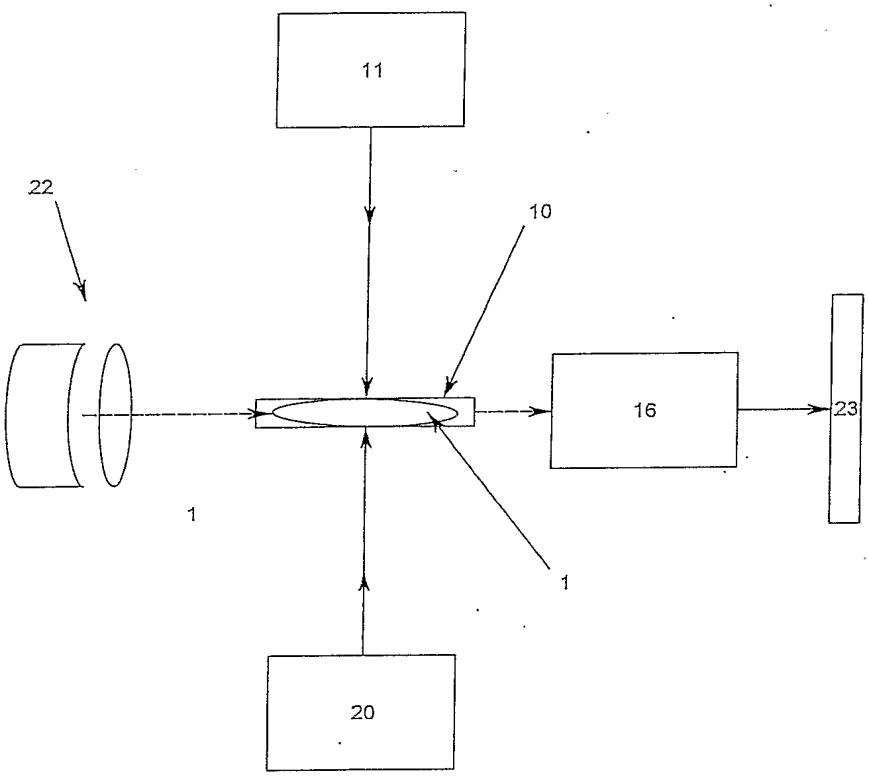

FIGURE 1B

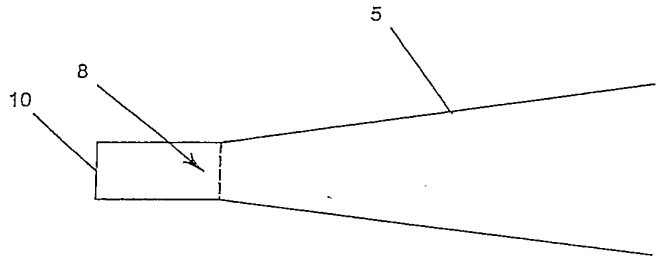

FIGURE 2

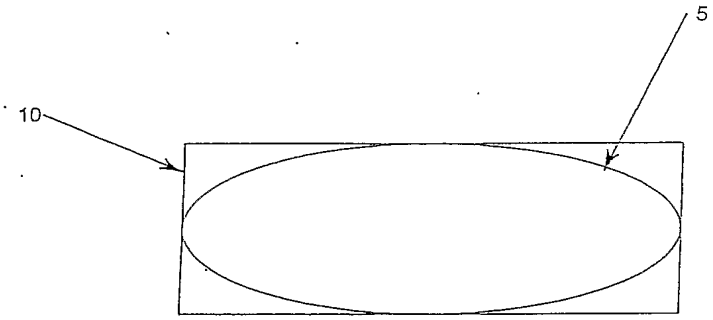

FIGURE 3